Vol. 13 April 1937 No. 4

Bulletin of the Agricultural Chemical Society of Japan.

ABSTRACTS

from

TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

On the Production of Aethylenoxide-α, β-dicarboxylic Acid by Mould. Part I. (pp. 241～246): By Kinichio SAKAGUCHI, Tatsuichiro INOUE and Seiji TADA. (The Agricultural Chemical Laboratory, Tokyo Imp. Univ. Received Feb. 9, 1937.)

Aethylenoxide-α, β-dicarboxylic acid or "fumaryl-glycidic acid" was first synthetized by Lossen from β-chlor- or brom-*d*, *l*-malic acid as its optically inactive form. Recently R. Kuhn and R. Zell (1) have succeeded the inactive acid to separate in active forms through its morphine salt.

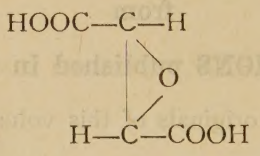
The authors have found that the acid was produced by a mould, isolated from a botanical specimen preserved in a very dilute formaldehyde solution, in amounts corresponding roughly with 10～15% of the total sugar utilized, when the mould was grown on the solution containing 10% of glucose: water to 1000 c.c.; glucose, 100 g.; nitrogen sources 1～5 g.; K_2HPO_4 and KH_2PO_4 each 0.2 g.; $MgSO_4 \cdot 7H_2O$ 0.01 g.; traces of NaCl and $FeCl_3$.

The acid is precipitated as its Ba-salt from the etherial extract of the metabolism solution. The mercurous salt of the acid, being insoluble in 5% solution of nitric acid, can also be used for the isolation of the acid from the etherial extract or even from the culture medium directly.

The acid obtained by the authors is colourless hexagonal plates, M. P. 179～180°C (corr.) (M. P. 180° Kuhn and Zell), the specific rotation, using a 10% aqueous solution, being $[\alpha]_D^0 - 100.°6$ ($[\alpha]_D^{18} - 100.°0$ Kuhn and Zell). It is easily soluble in water, ethyl alcohol, aceton and ether but difficulty in benzene, or petroleum ether. The acid does not reduce Fehling's solution nor has any other aldehydic and ketonic properties. It resembles *d*-tartaric acid in various tests but differs from the latter against Fenton's(2) and Furth and Herrmann's(3) reactions. It is easily hydrolysed to inactive mesotartaric acid and partly to *d*-tartaric acid by boiling its aqueous solution. By the

addition of hydrochloric acid the laevorotatory monochlormalic acid is obtained. Elementary analysis gave: C 36.37, 36.31%; H 2.96, 3.13% N negative; molecular weight 128, 130 by Rast's method, 132 from the titration number as a dibasic acid, while $C_4H_4O_5$ requires C 36.28%; H 3.06%; molecular weight 132.0.

According to Kuhn and Zell, the acid, which has the properties as above mentioned, corresponds to the trans form i.e.



Beside fumaryl glycidic acid, mannitol and several other organic acid such as succinic, citric and gluconic acids are also produced in various amounts.

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(2) Fenton: Z. f. anal. Chem. **21**, 123.
(3) O. Furth u. H. Herrmann: Bioch. Z. Bd. 280, Heft 56 (1935).

Studies on Alcohol Manufacture from Jerusalem Artichoke. Part I.—On the Saccharification of Jerusalem Artichoke. The Acid Hydrolysis (I). (pp. 247～261): By Toshinobu ASAI (The Chemical Laboratory, Morioka Agricultural College, Japan, Received Jan. 20, 1937.)

The saccharification of Jerusalem Artichoke with sulphuric acid was investigated. All experiments were carried out in the concentration under which about 10%～13% of maximum formation of sugars could be expected, i. e., five parts of acid to dried Jerusalem Artichoke and 30% of it to wet or fresh one.

The results obtained are summarized as follows:—

1) Inulin was converted into fructose with very ease. Under ordinary pressure, adding ten parts of 0.8% sulphuric acid and heating for 30 minutes at 100°C, the saccharification was almost complete (95.1%).

2) In the case of dried Jerusalem Artichoke the saccharification was completely carried out under the condition of 1.5% sulphuric acid was used and was heated at 100°C for 60 minutes, prolonged heating or higher concentration of the acid caused the decomposition of sugars formed. Working at high pressure the optimum condition was as follows: a) With 0.5% sul-

phuric acid, 30～50 minutes cooking under 30 lbs pressure. b) With 0.5% sulphuric acid, 20 minutes cooking under 40 lbs pressure. 50 lbs pressure caused sugar decomposition to a significant degree.

3) Before steaming of the materials in order to effect the destruction of the cell walls or to effect the conversion of inulin to dissolved state was also investigated and was confirmed that the treatment lowers the degree of cooking pressure and the duration of heating of acid saccharification. For example, in the case of direct cooking under 30 lbs pressure the highest saccharification value was obtained when 0.5% sulphuric acid was used and heated for 30～50 minutes, while the before treatment shortened the cooking time to 15～20 minutes in the same condition.

4) Dried Jerusalem Artichoke was far easily saccharified compared with dried potato or dried sweet potato. The saccharification ratio of the three materials was comparatively obserbed and it was ascertained that the dried Jerusalem Artichoke showed 100% of its ratio while in the dried potato only 17～25% of it and in the dried sweet potato only 27～42% of it were found.

5) Lastly, the wet Jerusalem Artichoke was taken for the acid saccharification. It was saccharified with smaller amounts of the acid and with less cooking pressure. Also the duration of cooking times decreased than the dried one, so it could be supplied for the alcohol manufacture in the harvest season with economical advantages.

Über die hypoglycämische Wirkung des Autolyse-Saftes der Hefe und Pilz, insbesondere über Kurven der Blutzuckererniedrigung. (S. 262～266): von Yoshio SHIZUME. (Agr. Chem. Laboratory. Tokyo Imp. Uuiv., Received Dec. 16, 1936.)

Chemical Studies on Japanese Coccidae. XV.—On the nitrogeneous and inorganic Substances of Sasakiaspis pentagona Tar. (pp. 267～274): By M. KAWANO and R. MARUYAMA. (Laboratory of Ohsaka Factory of Sankyo Co. Ltd., Received Dec. 10, 1936.)

On the Koji-amylase. Part VI.—Formation of Amylase, Maltase and Protease during Cultivation of Saké-koji. By Yuzo TOKUOKA. (Tsunekichi Okura Brewery, Received Jan. 25, 1937.)

Methods of preparation of enzyme solution from Saké-koji were discussed

with different temperatures, periods of extraction and various concentrations of salt solution. A considerable destruction of enzyme was observed during long period of extraction (more than 10 hours) or at high temperature about 30°, while any noticeable difference in the amount of extraction of enzyme was not pointed out with the concentrations of salt solution between 1 to 6%.

Therefore, in the present experiments, Saké-koji was extracted with 1% NaCl solution for 3 to 5 hours at room temperature, for the preparations of enzyme solution.

The relative proportion of Amylase (both dextrinising and saccharifying powers), maltase and protease, was found to be constant at any stage of cultivation of Saké-koji, while the rate of formation of Amylase was noticeably different with various kinds or stages of Saké-koji.

On the Koji-amylase. Part VII.—The Effects of Temperature for Cultivation and of Degree of Polishing of Rice on the Formation of Amylase, Maltase and Protease in Saké-koji. (pp. 281～285): By Yuzo Tokuoka. (Tsunekichi Okura Brewery, Received Feb. 22, 1937.)

For enzyme solutions, various kinds of Saké-koji were extracted with NaCl solution and diluted with so much water as to reveal the same dextrinising power.

When relative activity of maltase, protease and saccharifying power was observed with the diluted enzyme solutions, any remarkable difference was not pointed out between various degrees (10, 20, 30 or 40%) of polishing of rice, however Saké-koji obtained from unpolished rice was found to contain greater amount of maltase.

Saké-koji was cultivated for the first 21 hourse in the same temperature at 30～32.2°, and then exposed at various temperatures for 24 hours during which maximum temperature reached to 38.3, 41.1, 44.4, 48.7 and 51.7° respectively.

It was found that enzymatic activities especially maltase power in the Saké-koji cultivated at high temperatures (48.7 and 51.7) was much inferior to ordinary koji cultivated at 30～45°.

Beiträge zur Kenntnis der aus Antyū (ein geistigen Getränke in Formosa) isolierten drei Milchsäurebildnern. (S. 286～294): von K. Sato und Y. Sizume. (Laboratory of Kotobukiya Brewery, Osaka, Received Dec. 16, 1936.)

Studies on the production of Mucilage by Bacteria. (I)— The Classification of Natto-bacillus. (pp. 295～304): By Yukichi Go and Seiji NAKAMURA. (Institute of Chemistry, Faculty of Science, Osaka Imperial University, Received Jan. 25, 1937.)

1) We have isolated 52 strains of Natto-bacillus from Natto produced in various districts of this Country.
2) These strains were classified into 13 varieties which were grouped in 2 subspecies according to the location of endospore.
3) We have compared their ability of mucilage production by measuring viscosity of culture medium of Soy-beans extract.

Distribution of Ascorbic acid in each Organ of Hen. (pp. 305～308): By Saichi MACHIDA and Toru SASAKI. (Chemical Laboratory, Tokyo Agricultural College. Received Feb. 12, 1937.)

On the Urease of Yeast. (A Preliminary Report). (pp. 309～313): By Kinichiro SAKAGUCHI and Yoshio SHIZUME. (The Agricultural Chemical Laboratory, Tokyo Imp. Univ., Received Feb. 9, 1937.)

It has known a long time that urea can serve yeasts as a source of nitrogen[1]. But neither the ureolytic action by the living yeast cells nor the enzyme responsible for it has hetherto been fully recognized. Thus Thomas[2], Sandberg[3] and Kiesel[4] have all failed to demonstrate the splitting of urea by various beer yeasts. The last author, however, found that a trace of ammonia is produced from urea by the autolysed Frohberg yeast which, according to H. Euler[5], is not sufficient to verify the urease in the yeast cells and is not generally accepted.

The present authors have found, out of 62 species of yeasts, 8 species which produce ammonia in the medium containing urea as a sole source of nitrogen. The ureolysis was also demonstrated with the autolysates of the yeasts in presence of toluol. On the method of the purification of yeast urease and the various conditions governing its activity must be studied in the further work.

EXPERIMENTAL.

Culture medium:—Urea, 1 g; Glucose, 2 g; K_2HPO_4, 0.05 g; $MgSO_4 \cdot 7H_2O$, trace; phenolphthalein, trace; dist. water 1000 c.c. (the P_H after the sterilization = 6.8).

Each 8 c.c. of the above medium is sterilized in test tubes for successive three days at a temperature as low as possible to avoid the thermal destruction of urea, and inoculated with the following species of yeasts from their Koji extract agar slant cultures.

The red coloration of the medium through the liberated ammonia is then observed. The quantities of ammonia formed are estimated by the distillation according to Wurster's method.

TABLE I.

	Urea in the original medium	Growth	Red coloration of the medium	the date of the coloration	Ammonia liberated		Urea decomposed	Urea decomp. / Total urea
					Control (not inoculated)	inoculated		
	g					g		%
Aspergillus niger	0.05	+	+	6	0	0.0098	0.0164	32.80
Torula rubescens	,,	+	+	3	0	0.0050	0.0084	16.80
Torula rubra A	,,	+	+	4	0	0.0022	0.0038	7.40
Torula rubra B	,,	+	+	3	0	0.0034	0.0057	11.40
Torula No. 604	,,	+	−	−	0	0.0003	0.0005	0.10
Mycoderma C	,,	+	−	−	0	0.0014	0.0023	4.60
A yeast from Soy beans	,,	+	+	8	0	0.0028	0.0047	9.50
Schizosacch. santaweniss	,,	+	−	−	0	0.0018	0.0030	6.00
Torulaspora Delbrücki	,,	+	−	−		0.0003	0.0005	0.10
Sake yeast No. 5	,,	+	−	−		0.0003	0.0005	0.10
Zygosacch. Barkeri	,,	+	−	−		0	0	0

The results obtained with the following species are all the same as that with Zygosacch. Barkeri above mentioned.

Zygosacch. mandshuricus, Z. bisporus, Z. salsus saccharosum II, Z. soya (Kikkoman), Z. soya (Higeta), Torula B, T. DIV, T. 14, T. G, Aging yeast of Sake A, B, C, D, Willia anomala, Willia anomala α, W. anomala Mb, W. saturnus, Beer yeast Frohberg, Saaz, Carlsberg, München, Basso beer yeast No. 1, Sacch. cerevisiae Rasse 776, Wine yeast Johannisberg, Albo II, Foller blanche de Cognac, Sauterne, Ungarn, Niersteiner, Charante fine champagne, Oppenheimer Goldberg, Heimersheimer Ruth, Wiltniger, Saccharomyces apiculatus, Hanseniaspora valbyensis, Pseudosacch. Mülleri, Debaryomyces Klöckeri, Endomyces Lindneri, Endomyces Hordei, Schizosacch. Hofberger, Sch. 39, 44, 46, Distillery yeast Miyamae, Dist. yeast Rasse II, Saccharomyces formosensis, Baker's yeast Fleischmann, Sankyo, Oriental.

In the following experiment 100 c.c. of the medium is used for each species.

TABLE II.

	Urea in 20 c.c. of the original medium	Ammonia formed			Urea decomp. / total urea added	
		control (after 7 days	after 3 days	after 7 days	after 3 days	after 7 days
	g					
Torula rubescens	0.2	0.0003	0.0036	0.0048	2.95	4.00
Torula rubra A	,,	,,	0.0008	0.0031	0.65	2.60
Torula rubra B	,,	,,	0.0017	0.0025	1.40	2.10
Mycoderma C	,,	,,	0	0	——	——
Torulaspora delbrücki	,,	,,	0	0	——	——
Schizosacch. santawensis	,,	,,	0.0006	0.0025	0.50	2.10
A yeast from Soy beans	,,	,,	0.0028	0.0042	2.35	3.50
Sake yeast No. 5	,,	,,	0	0	——	——
Sacch. cereviciae Frohberg	,,	,,	0	0	——	——

The species which were found to have the ureolytic ability in the above experiments are then cultivated in Koji extract for 4 days and then precipitated by centrifugal separator.

The yeasts thus obtained, being washed with steril water several times, are autolysed at 40°C for 2 days in the presence of toluol. To the autolysates, from which the cells of yeasts were completely removed by centrifuging, 20 c.c. of the 1% solution of urea and a few drops of toluol are added and kept at 40°C for 5～8 hours. The quantities of ammonia produced are as follows.

	Urea in 20c.c. of the sol.	Ammonia produced		Urea decomposed	Urea decomp. / Urea originally present
		Control (Autolysate)	Autolysate+ sol. of urea		
	g				
Torula rubescence	0.2	0.0	0.0009	0.0014	0.70
T. rubra A	,,	0.0	0.0007	0.0012	0.60
T. rubra B	,,	0.0	0.0010	0.0017	0.85
A yeast from Soy beans	,,	0.0003	0.0017	0.0028	1.40

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On the Koji-amylase. Part VIII.—The Fluctuation of Amylase during the Cultivation of Moto, Yeast Mashes. (pp. 313～317): By Yuzo TOKUOKA. (Tsunekichi Okura Brewery, Received Feb. 22, 1937.)

The fluctuation of amylase during the cultivation of yeast mashes for Saké manufacture, was found very similar to that of Saké mashes (see Part II).

In the early stages of cultivation, salt solution revealed very remarkable effect on the extraction of amylase, while any noticeable difference in amylase power of between aqueous and salt extracts of yeast mash, was no more observed at the latter stages when steamed rice being existed was almost dissolved out.

The total amount of amylase was again decreased during the cultivation owing to increase of temperature, production of acid and alcohol.

Thus the above, it was again ascertained that steamed rice revealed a remarkable adsorption of Koji-amylase.

The Micro-determination of Potassium and Natrium in Milk. (pp. 318～322): By Masayoshi SATO and Kiichi MURATA. (Zootechnical Institute, Hokkaido Imperial University, Sapporo, Japan. Received March, 15, 1937.)

Five cc. of Milk is placed in a 25 cc. volumetric flask, then added 0.5 cc. of 3% acetic acid, 5 cc. of 96% alcohol drop by drop. Allowing to stand for at least 3/4～1 hour, and the volume made up to 25 cc. with distilled water, and then filtered with a dry filter paper.

These protein-free filtrates are used for determinating of potassium and natrium in milk.

Potassium ; —The potassium is precipitated as potassium cobalti-nitrite in the above protein free filtrate by adding an excess of the natrium cobalti-nitrite reagent. The precipitate is dissolved with nitric acid and then reduced with the addition of dimethyl glyoxim ahd natrium sulphide.

The color thus developed is compared with the color of the standard.

Natrium ; —The Natrium is precipitated as Natrium pyroantimonate in the protein free filtrate by adding an excess of the potassium pyroantimonate reagent.

The precipitate is washed with dilute alcohol, and dissolving by concentrate hydrochloric acid is titrated wirh 0.01 *N.* natrium thiosulphate by iodometric method.
